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Association between exposure to secondhand smoke and telomere length: cross-sectional study of 1,303 non-smokers

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Abstract

Background

Both active smoking and secondhand smoke (SHS) are important risk factors for many age-related diseases. Active smoking is associated with shortened telomere length. However, whether SHS accelerates telomere attrition with age is uncertain. The aim of this study was to examine the association between SHS exposure and shortening by age of leukocyte telomere length among adult non-smokers.

Methods

We undertook a cross-sectional study of the association between self-reported levels of SHS exposure and telomere length shortening per annum on a subgroup of participants from the Scottish Family Health Study. Inclusion was restricted to non-smokers aged ≥ 18 years who had provided self-reported overall usual SHS exposure (total hours per week) and blood samples for telomere analysis. Linear regression models were used to compare T/S ratio by age according to SHS exposure.

Results

Of the 1,303 eligible participants, 779 (59.8%) reported no SHS exposure, 495 (38.0%) low exposure (1-19 hours per week) and 29 (2.2%) high exposure (≥ 20 hours per week). In the univariate linear regression analyses, relative telomere T/S ratio declined with increasing age in all exposure groups. Telomere length decreased more rapidly with increasing age among those with high exposure to SHS (adjusted coefficient -0.019, 95% CI -0.031- -0.007) when compared with both those with no exposure to SHS (adjusted coefficient -0.006, 95% CI -0.008- -0.004) (high vs no SHS: $p=0.010$) and those with low exposure to SHS (adjusted coefficient -0.005, 95% CI -0.007- -0.003) (high vs low SHS: $p=0.005$).

Conclusion

Our findings suggest that high SHS exposure may accelerate normal biological ageing and support efforts to protect the public from SHS exposure. Further studies on relevant mechanisms should be conducted.

MeSH terms

telomere length, secondhand smoke, biological ageing

Key messages

- Telomere length decreased more rapidly with increasing age among those with high exposure to SHS when compared with both those with no exposure to SHS and those with low exposure to SHS.
- Our findings suggest that high SHS exposure may accelerate normal biological ageing.
- Our study supports the efforts to protect the public from SHS exposure.

Introduction

Telomeres are nucleoprotein complexes at the end of the chromosomes, which protect the chromosomal DNA from deterioration and end-to-end fusion.¹ Telomeric DNA comprises stretches of repetitive (TTAGGG)_n sequence which is eroded with each cycle of cell division as part of the end replication problem.² Telomere attrition normally limits cells to a fixed number of divisions and eventually results in the loss of genetic information from the cell's chromosomes with further divisions.^{1, 3} Environmental factors, including cigarette smoking, that elevate levels of oxidative stress can accelerate the attrition and therefore biological ageing.⁴⁻⁶ Previous studies have demonstrated that shorter telomere length is associated with common age-related diseases, including cardiovascular disease and chronic obstructive pulmonary disease, and a shorter life span, through mechanisms involving oxidative stress associated with cigarette smoking.⁷⁻⁹ Several studies have suggested that individuals with shorter telomere length have increased risk of developing certain types of cancer.^{10, 11}

Both active cigarette smoking and secondhand smoke (SHS) are important risk factors for many age-related diseases,^{12, 13} cancer,^{14, 15} and mortality,^{16, 17} and are associated with increased oxidative stress, chronic inflammation and endothelial dysfunction¹⁸. Previous studies on active smoking have demonstrated a dose-response relationship between cumulative lifetime exposure to cigarette smoking and telomere length attrition.^{8-10, 19} Studies also now suggest that prenatal tobacco exposure is associated with shortened telomere length in children²⁰ and newborn babies²¹.

SHS is a mixture of side-stream smoke from the burning cigarette tip and mainstream smoke exhaled by the smoker. It contains at least 250 harmful chemicals including carcinogens, carbon

monoxide and tar (www.who.int/mediacentre/factsheets/fs339/en/). Side-stream smoke, in particular, contains higher concentrations of toxic gases and small (<2.5 µm), respirable particles than mainstream smoke.²²⁻²⁵

To our knowledge, very few studies have examined the association between SHS exposure in adulthood and leukocyte telomere length attrition.^{26, 27} In one study, the primary aim was to examine the relationship between levels of traffic pollution and telomere length. The investigators reported shorter telomere length in traffic officers than indoor office workers. The study was underpowered for the secondary aim of studying the relationship between telomere length and SHS exposure among the sub-group of never smokers and it found no significant association.²⁶ To examine the association between levels of SHS exposure and telomere attrition per year of age among adult non-smokers, we conducted a cross-sectional study using a subgroup of individuals from the Scottish Family Health Study who had already had telomere assays performed as part of a previous study of biological aging.

Methods

Data sources

Generation Scotland: the Scottish Family Health Study (GS:GFHS) is a family-based, cross-sectional study of the general population, with a specific focus on cardiovascular risk factors and disease (<http://www.generationscotland.org>). Probands aged between 35 and 55 years were randomly selected for invitation from the records of general practitioners based in Glasgow and Dundee, Scotland. Between 2006 and 2011, 7,953 probands were recruited along with 16,007 consenting first degree relatives aged ≥ 18 years; producing a total of 23,960 participants.²⁸ All participants completed a questionnaire that provided information on demographics (including age, sex and postcode of residence) and lifestyle (including smoking status, and number of hours of exposure per week to SHS). Trained staff measured height and weight and collected blood samples from consenting participants.

Ethical approval for the GS:SFHS was obtained from NHS Tayside Committee on Medical Research Ethics (REC Reference Number: 05/S1401/89). GS:SFHS has been granted Research Tissue Bank status by the Tayside Committee on Medical Research Ethics (REC Reference Number: 10/S1402/20) providing generic ethical approval for a wide range of uses within medical research. Permission to use the GS:SFHS data and access to the blood samples was provided following review by the GS:SFHS Access Committee.

This study used existing data on a sub-group of 1,779 SFHS participants, randomly selected to participate in a study on ageing. DNA was extracted from peripheral blood leukocytes using Maxwell automated purified system (Promega, WI, USA). Telomere length in the DNA

samples were determined by quantitative-polymerase chain reaction (Q-PCR) blindly using a Roche Light Cycler LC480 (Roche Diagnostics, Indianapolis, Indiana, USA).²⁹

Analyses were performed in triplicate for each sample using a single-copy gene amplicon primer set (acidic ribosomal phosphoprotein, 36B4) and a telomere-specific amplicon primer set.³⁰ A cut-off 0.15 for the standard deviation (SD) of the threshold cycle (Ct) for sample replicates was used as a quality control parameter for the amplification. The samples were reanalysed if a SD above 0.15 was encountered. The average SD across plates was 0.07. Relative telomere length was estimated from Ct scores using the comparative Ct method when telomere and control gene assays yielded similar amplification efficiencies. The ratio of telomere repeat copy number to single copy gene number (T/S) ratio in experimental samples relative to a control sample DNA was determined. This normalised T/S ratio was defined as the estimate of relative telomere length (relative T/S). The inter-assay variation was tested by comparing the relative T/S estimates for positive controls on every assay plate. The average inter-assay coefficient of variance was 0.58% for telomere length and 0.23% for 36B4.^{29, 31, 32}

Inclusion criteria and definitions

Inclusion was restricted to self-reported non-smokers (never or ex-smokers) aged ≥ 18 years who had provided blood samples for telomere analysis. Area-based socioeconomic status was measured using the Scottish Index of Multiple Deprivation (SIMD) (www.scotland.gov.uk/topics/Statistics/SIMD). There are 6,505 datazones based on the postcode of residence, with a mean population of 800. The SIMD for each datazone is derived from information on income, employment, health, education, housing, crime and access to services and is grouped into deprivation quintiles for the Scottish general population, ranging from 1 (most deprived) to 5 (least deprived). Body mass index (BMI) was defined as: normal weight ($<25 \text{ kg/m}^2$), overweight ($25\text{-}30 \text{ kg/m}^2$) and obese ($\geq 30 \text{ kg/m}^2$). Levels of usual, overall

SHS exposure were self-reported and categorised into: no exposure, low exposure (1-19 hours per week) and high exposure (≥ 20 hours per week).

Statistical analyses

The characteristics of this study population were summarised using frequencies and percentages. The differences between the exposure groups were assessed using chi-square tests. Linear regression models were used to compare the coefficients (slopes) in telomere T/S ratio by age between different exposure groups. Statistical significance was defined as $p < 0.05$ in two-sided tests. All statistical analyses were undertaken using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

Results

Of the 1,779 SFHS participants, 1,721 had provided adequate blood samples for telomere assays. Among these, 1,433 were self-reported non-smokers. Of the non-smokers, 1,303 provided self-reported SHS exposure status and therefore comprised this study population. Among these, 861 (66.1%) were men and 442 (33.9%) women, 846 (64.9%) classified themselves as never smokers and 457 (35.1%) as ex-smokers; of the latter, 404 (88.4%) had quit smoking for at least one year. Among the 1,303 eligible participants, 779 (59.8%) reported no SHS exposure, 495 (38.0%) low exposure (1-19 hours per week), 29 (2.2%) high exposure (≥ 20 hours per week) (Table 1). Participants with high SHS exposure were older in age (p for trend=0.025), lived in more socioeconomically deprived areas (p for trend<0.001), and were more likely to be obese (p for trend=0.012) when compared with participants with no SHS exposure.

In the univariate linear regression analyses, relative telomere T/S ratio declined with increasing year of age in all exposure groups. Telomere length decreased more rapidly with increasing age among those with high exposure to SHS (unadjusted coefficient -0.015, 95% CI -0.025- -0.005, $p < 0.001$) when compared with both those with no exposure to SHS (unadjusted coefficient -0.006, 95% CI -0.007- -0.004, $p < 0.001$) (high vs no SHS: $p = 0.047$) and those with low exposure to SHS (unadjusted coefficient -0.006, 95% CI -0.008- -0.004, $p < 0.001$) (high vs low SHS: $p = 0.047$). After adjustment for sex and deprivation quintiles, the differences were more statistically significant: reduction per year of age among those exposure to high levels of SHS (adjusted coefficient -0.019, 95% CI -0.031- -0.007, $p = 0.003$) compare with those with no exposure (adjusted coefficient -0.006, 95% CI -0.008- -0.004, $p < 0.001$) (high vs no SHS: $p = 0.010$) and low exposure (adjusted coefficient -0.005, 95% CI -0.007- -0.003, $p < 0.001$) (high vs low SHS: $p = 0.005$) (Figure 1). After further adjustment for BMI category, telomere length

still decreased more rapidly among participants with high exposure to SHS (adjusted coefficient -0.017, 95% CI -0.030- -0.004, $p=0.011$) compared with no exposure group (adjusted coefficient -0.006, 95% CI -0.008- -0.004, $p<0.001$) (high vs no SHS: $p=0.024$) and with low exposure (adjusted coefficient -0.005, 95% CI -0.007- -0.003, $p<0.001$) (high vs low SHS: $p=0.014$).

Discussion

Our findings suggest that high SHS exposure may accelerate normal biological ageing, as measured by leukocyte telomere length. Telomere attrition has been demonstrated to be associated with many age-related diseases including cardiovascular disease phenotypes,^{7, 33, 34} chronic obstructive pulmonary disease,⁹ and certain types of cancer^{10, 11}. Therefore, it may be one of the mechanisms by which SHS is associated with these outcomes.

Both active smoking and SHS exposure increase inflammation, thrombosis and oxidative stress.^{18, 35} Several studies have demonstrated an association between active smoking and telomere attrition. In a cross-sectional study of 1,122 healthy women aged 18-76 years, the investigators showed that never smokers had longer age-adjusted telomeres than former smokers, and both had longer telomeres than current smokers. Among current smokers, there was a dose relationship whereby each pack-year smoked equated to an additional 5 base pair (bp), or 18% of the average annual loss in age-adjusted telomere length.⁸ In a cross-sectional study of 50 life-long smokers and 26 never-smokers aged ≥ 55 years, cumulative active smoking exposure was found to be associated with relative telomere length, with a dose-response relationship whereby telomere length decreased with the increasing pack-years ($r=-0.45$, $p<0.001$).⁹ In the Health Professionals Follow-up Study, researchers examined the association between telomere length and risk of bladder cancer among men aged 40-75 years in the USA. They conducted a case control study of 123 men with bladder cancer and 125 men without and demonstrated a similar dose-response relationship between relative telomere length and pack-years of active smoking among control smokers ($r=-0.25$, $p=0.02$).¹⁰

Most of the research into the effect of passive exposure to tobacco has focused on prenatal exposure. A cross-sectional study examined the association between prenatal tobacco exposure and salivary relative telomere T/S ratio among 101 African American children aged 4-14 years. Among these children, 18.8% had prenatal tobacco exposure in their home, and 6.8% had mothers who smoked during pregnancy. Children who were exposed to smoke during pregnancy had shorter mean telomere length than those not exposed (mean \pm SD 6.4 \pm 1.5 vs. 7.5 \pm 2.3; χ^2 test $p < 0.05$).²⁰ A prospective study, undertaken in Florida, examined the relationship between intrauterine tobacco exposure and fetal telomere length among 86 pregnant women aged ≥ 18 years who were divided into non-smokers, passive smokers and active smokers. Fetal telomere length was determined using umbilical cord blood at birth and measured as T/S ratio. Smoking exposure was measured using both a questionnaire and a salivary cotinine test. They used simulation analysis to derive precise estimates and found a dose-response pattern, with shortest fetal telomere lengths among the offspring of active smokers followed by passive smokers and longest among non-smokers.²¹

In contrast, very few studies have been published to date on the association between SHS exposure in adulthood and telomere length.^{26, 27} One cross-sectional study, conducted in the USA, examined 77 traffic officers exposed to high levels of traffic pollutants and 57 office workers as controls. Among the office workers, ever smokers had shorter telomeres than never smokers (geometric mean 1.17, 95% CI 1.10-1.25 for ever smokers vs 1.33, 95% CI 1.20-1.48 for never smokers, $p = 0.04$). The study reported no significant association between SHS exposure and telomere length. They also reported no significant association with either number of cigarettes smoked per day or pack-years.²⁶ The study was underpowered to study the effects of SHS exposure; of the 66 study participants who had never smoked, 28 reported exposure to SHS. In comparison, our study comprised 1,303 non-smokers of whom 524 reported SHS

exposure. This provided sufficient power to not only examine the overall effect of SHS exposure but also to explore whether there was evidence of a dose relationship across different levels of SHS exposure. Within the limits of comparing only three ordinal categories, our findings do not support a dose-relationship across the range of SHS. This is consistent with previous studies. A cohort study published in 2016, examined the association between smoking exposure, both active and secondhand, and telomere length among 6,456 adult participants in NHANES.²⁷ The investigators found no overall association between SHS and mean leukocyte telomere length when they treated the former as a continuous variable based on: self-reported time of exposure at home; self-reported time of exposure at work, and serum cotinine. Using quantile regression they found a significant inverse association between cotinine and leukocyte telomere length in the highest decile of telomere length only; confirming a non-linear relationship between SHS exposure and telomere length.²⁷ Another NHANES study provided similar findings based on disease end-points. Agarwal et al. reported no association between cotinine and peripheral arterial disease when cotinine was treated as a continuous variable.³⁶ However, re-analysis using quintiles demonstrated significantly increased risk of peripheral arterial disease among participants with the highest 15% of cotinine concentrations, suggestive of a threshold effect.³⁶ In order to maximise statistical power, we included ex-smokers with never smokers, as non-smokers, in this study but the majority of the ex-smokers had not smoked for more than a year.

In the regression models, we were able to adjust for sex and deprivation quintile, and the association increased in magnitude. This suggests that individuals exposed to both socioeconomic deprivation and SHS exposure may be at particularly high risk of premature biological ageing. As with any non-randomised study, residual confounding is possible. For example, we were not able to adjust for physical activity data which were missing on a large

proportion of participants. However, a systematic review by Mundstock et al. concluded that there is currently insufficient good quality evidence to establish an association between physical activity and telomere length.³⁷ We did not have access to an objective measurement of SHS exposure, and therefore had to rely on self-report. Self-report is commonly used in large population studies because questionnaires are cheaper and easier to administer than biomarkers. Unlike biomarkers, questionnaires can be used to ascertain usual, or long-term, exposure and exposure in specific sites as well as overall exposure.³⁸ Cotinine, the primary metabolite of nicotine, is now the most commonly used biomarker of SHS. It can be measured in the saliva, blood or urine but only measures recent, overall exposure and concentrations vary, not only with nicotine exposure, but also individual nicotine metabolism, which changes with age and when pregnant.³⁹⁻⁴² Hair is the only tissue that can be used to measure long-term exposure but is rarely used.⁴³ Biomarkers are more accurate and not subject to potential bias⁴⁴; however, systematic errors are unlikely in this study since telomere length was unknown to participants and its measurement postdated self-report of SHS. Willemssen et al. demonstrated a moderate correlation ($r=0.65$, $p<0.001$) between self-reported average exposure to SHS at work and nicotine concentrations.⁴⁵

Since the study was cross-sectional, temporal relationship cannot be established; however, reverse causation is unlikely to be plausible. Plausible mechanisms include cumulative oxidative stress-mediated damage and the stimulation of inflammation. Since the implementation of the comprehensive smoke-free legislation in Scotland in 2006, exposure to SHS has declined in all public places covered by the legislation.⁴⁶ This may be a possible explanation that in our study the number of participants who reported high exposure to SHS was small. The results of this study should be incorporated in future large-scale studies, ideally with access to objective measurements of SHS exposure, including repeat measures over time,

and sufficient statistical power to study never smokers in isolation to determine whether there is a dose-response relationship.

Conclusion

Our study adds to the relatively sparse literature on SHS exposure and biological ageing. Our findings suggest that high levels of SHS exposure may accelerate the shortening of telomeres that occurs naturally with age, and reinforces the importance of protecting the general public from exposure to SHS.

Contributorship Statement

JPP had the original concept. All of the authors agreed the methodology. LL and DFM performed the statistical analyses. All authors interpreted the results. LL drafted the manuscript. All authors fed back comments. All authors read and approved the final manuscript.

Conflicts of interest

The authors declared no conflicts of interests.

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Table 1. General characteristics of the 1,303 non-smokers

	Total hours per week to secondhand smoke			P value*
	0 (n=779)	1-19 (n=495)	≥ 20 (n=29)	
Age group, n (%)				<0.001
<45 years	368 (47.2)	327 (66.1)	20 (69.0)	
45-59 years	193 (24.8)	98 (19.8)	5 (17.2)	
≥60 years	218 (28.0)	70 (14.1)	4 (13.8)	
Missing	0	0	0	
Gender, n (%)				0.190
Male	529 (67.9)	312 (63.0)	20 (69.0)	
Female	250 (32.1)	183 (27.0)	9 (31.0)	
Missing	0	0	0	
Deprivation, n (%)				<0.001
1 (least deprived)	297 (38.1)	164 (33.1)	4 (13.8)	
2	211 (27.1)	123 (24.9)	6 (20.7)	
3	99 (12.7)	73 (14.8)	5 (17.2)	
4	52 (6.7)	64 (12.9)	8 (27.6)	
5 (most deprived)	50 (6.4)	44 (8.9)	4 (13.8)	
Missing	70	27	2	
BMI category				0.024
<25 kg/m ²	327 (42.0)	231 (46.7)	7 (24.1)	
25-30 kg/m ²	312 (40.1)	170 (34.3)	12 (41.4)	
≥30 kg/m ²	135 (17.3)	89 (18.0)	10 (34.5)	
Missing	5	5	0	
Smoking status, n (%)				<0.001
Never smokers	496 (63.7)	336 (67.9)	14 (48.3)	
Ex-smokers stopped smoking > 1 year	272 (34.9)	122 (24.6)	10 (34.5)	
Ex-smokers stopped smoking ≤ 1 year	11 (1.4)	37 (7.5)	5 (17.2)	
Missing	0	0	0	

BMI body mass index;

*chi-square tests

Figure 1. Change in telomere length T/S ratio per year of age and levels of secondhand smoke exposure among non-smokers (adjusted for sex and deprivation)

